Risk of Spontaneous Preterm Birth is Associated With Common Proinflammatory Cytokine Polymorphisms

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Background: Preliminary data suggest that common genetic variation in immune response genes can contribute to the risk for spontaneous preterm birth and possibly small-for-gestational age (SGA).

Methods: We investigated the relationship of polymorphisms in 6 cytokine genes associated with inflammation—interleukin (*IL)1α, IL1β, IL2, IL6*, tumor necrosis factor (*TNF*), and lymphotoxin α (*LTA)*—with spontaneous preterm and SGA birth in a nested case–control study drawn from a prospective pregnancy cohort. Women were recruited between 24 and 29 weeks' gestation at the Wake County and University of North Carolina, Chapel Hill obstetric clinics between February 1996 and June 2000. We inferred haplotypes using the EM algorithm and the Bayesian method, PHASE. We then compared haplotype frequency distributions and implemented semi-Bayesian hierarchical logistic regression analyses to obtain odds ratio (OR) estimates and 95% confidence intervals (CIs) for each polymorphism.

Results: Two haplotypes spanning the *TNF/LTA* genes were associated with increased risk for spontaneous preterm birth in white subjects (for the AGG haplotype, OR = 1.5 [95% CI=0.8–2.6]; for the GAC haplotype, 1.6 [0.9–2.9]). Additionally, carriers of the GAG haplotype were found to have decreased risk of spontaneous preterm birth (0.6; 0.3–1.0). The *TNF(−488)A* and *LTA(IVS1−82)C* variants, constituents of the AGG and GAC haplotypes respectively, were also strongly associated with increased risk of spontaneous preterm birth.

Conclusions: Our results suggest that common genetic variants in proinflammatory cytokine genes could influence the risk for spontaneous preterm birth. Selected *TNF/LTA* haplotypes were associated with spontaneous preterm birth in both African-American and white subjects. Our data do not support an inflammatory etiology for SGA.

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There is increasing evidence that infection and the inflammatory response contribute to the etiology of spontaneous preterm birth.[1–4] Genetic susceptibility factors in immune response genes have been investigated, particularly cytokine polymorphisms known to be proinflammatory.[5–14] To date, the most frequently investigated single nucleotide polymorphisms (SNPs) include tumor necrosis factor (*TNF)(−488*), interleukin (*IL)1β(+3954)*, and *IL1β(−511*), each of which appears to be associated with alterations in cytokine expression. Although previous studies have suggested associations with preterm birth, replication of results has been controversial, in part due to issues of study design (ie, insufficient power, bias in selection of cases or controls, and population stratification).

The genomic revolution has generated a catalog of common genetic variants and provided an opportunity to directly examine their contribution to complex conditions, such as spontaneous preterm and small-for-gestational age (SGA) birth.[15] Many researchers advocate conducting primary investigations with haplotypes rather than SNPs be-

cause haplotypes can span larger regions of a potential gene of interest.[16] Haplotypes are derived from blocks of DNA known to be in linkage disequilibrium. Because a haplotype is an extended region of linked variants that are inherited as a unit from a single chromosome, allelic variants that travel together on the same haplotype can be analyzed simultaneously (known as *cis* interactions).[16] Additionally, a critical benefit of haplotypes is in examining haplotype diversity and frequency across populations (known as "transracial mapping"). In the case of common conditions, such as preterm and SGA births, this approach may help identify variants common across populations that are likely to be of etiological importance.[16]

We examined the relationships of common polymorphisms in proinflammatory cytokines with spontaneous preterm birth and SGA in a cohort of women enrolled in a prospective pregnancy study. In this report, we will describe the relationship among 10 polymorphisms on proinflammatory cytokine genes *IL1α*, *IL1β, IL2, IL6, TNF*, and lymphotoxin α (*LTA*), bacterial vaginosis infection, and spontaneous preterm birth and SGA. The relationship of anti-inflammatory cytokine polymorphisms with spontaneous preterm birth and SGA is described in a companion report.[17]

METHODS

Study Design

The Pregnancy, Infection, and Nutrition cohort study enrolled women between 24 and 29 weeks' gestation at Wake County Human Services Department and the Wake Medical Center/Wake AHEC from February 1996 through June 1998 and at the University of North Carolina, Chapel Hill obstetric clinics from August 1995 through June 2000.[18] We collected genital tract swabs and blood from women at the intake visit. Study exclusions are detailed elsewhere.[18] From the larger cohort, a nested preterm case–control sample was selected for DNA extraction, genotyping, and analysis, to yield an approximate 1:1.5 case:control ratio. An additional 100 women who were neither preterm cases nor randomly selected controls delivered an SGA infant and consented to genetic analysis. These women were added to the SGA analysis (Fig. 1). Characteristics of our study population can be found in Appendices 1 and 2. (All appendices are available with the electronic version of this article.)

Gestational age was assigned by early ultrasound measurements completed before 22 weeks' gestation. In the absence of early ultrasonography, last menstrual period date was used. Preterm birth was dichotomized as birth before 37 completed weeks of gestation. Spontaneous preterm birth included those with spontaneous membrane rupture occurring at least 4 hours before the onset of labor, and births after spontaneous labor within 4 hours of membrane rupture. Study obstetricians reviewed records of cases to determine preterm clinical presentation. For births with ambiguous information in the charts and for a 10% random sample of all preterm births, further review was conducted by an obstetrician who was unaware of the initial categorization. Infants were classified as SGA if they fell below the 10th percentile of weight for gestational age, stratified by race, sex, and parity.

Bacterial Vaginosis Assessment

Participants had genital tract swabs collected during a routine pelvic examination between 24 and 29 weeks' gesta-

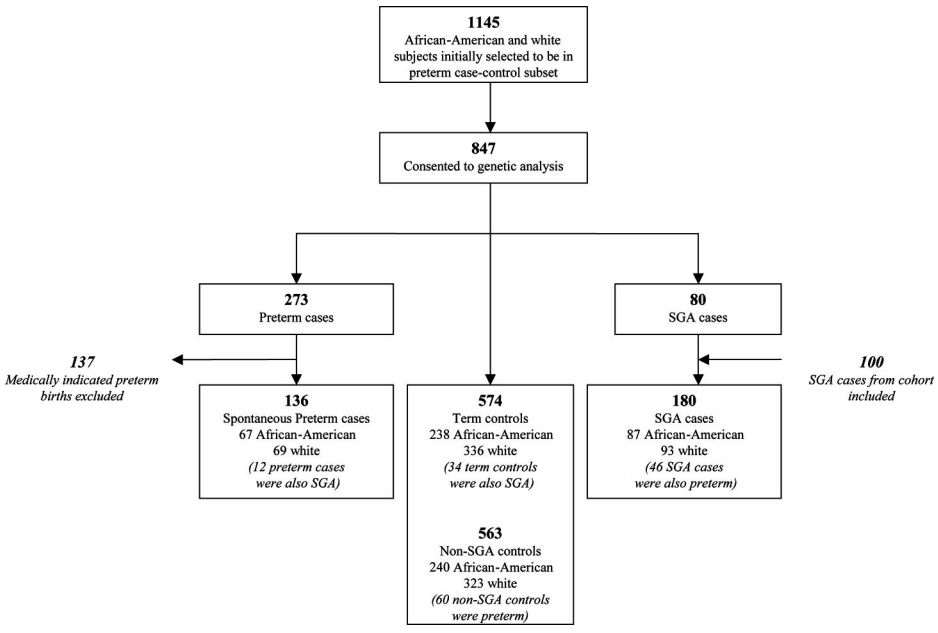

FIGURE 1. Sample size for spontaneous preterm birth and SGA analysis of African-American and white women. The study population was limited to African-American and white women who consented to genetic analysis. The initial case–control subset was selected on the basis of preterm status. Additional SGA cases were included from the cohort when they were not already selected as a preterm case or control. In the SGA analysis, 58 women who were missing SGA status and 146 women who were preterm but not randomly selected as control subjects were excluded.

tion. Dacron swabs were wiped against the lateral vaginal side wall and then rolled onto a glass microscope slide and allowed to air dry. All slides were gram-stained, counterstained with safranin and evaluated for 3 morphotypes of bacteria. A detailed description of this assessment can be found elsewhere.[19] Briefly, a score was determined by summing points assigned to each morphotype identified. Designation of bacterial vaginosis was made for vaginal flora scores of 7–10, whose vaginal pH was greater than 4.5.

DNA Extraction and Genetic Analyses

DNA was extracted from peripheral blood samples collected at the intake visit using the ABI automated DNA extractor. Genotyping for selected polymorphisms was performed using TaqMan for allelic discrimination (Table 1).[20] A 10% random sample, distributed randomly across plates, was assayed in duplicate for quality control.

Analytic Methods

Deviations from Hardy–Weinberg equilibrium were examined in the control series stratified by race. Loci that violated Hardy–Weinberg in control subjects were excluded from haplotype analyses. Linkage disequilibrium, within strata of race and case-status, was assessed for SNPs that occurred on the same chromosome; pairwise comparison measures were calculated in Arlequin.[21] Linkage disequilibrium was assessed for polymorphisms assuming known gametic phase using D′. D′ with an absolute value of 1 indicates loci are in complete linkage. Linkage disequilibrium also was calculated assuming unknown gametic phase using likelihood ratio tests that compared the likelihood of the sample under the hypothesis of no association between loci to the likelihood of the sample allowing the loci to be associated.[22] Population haplotype frequencies in case and control groups separately and combined were estimated using the Excoffier and Slatkin EM algorithm.[23] Frequency distributions were then compared using an omnibus likelihood ratio test.[24] This test computes a haplotype frequency likelihood ratio statistic comparing haplotype frequency likelihoods for cases and controls separately versus combined. A null distribution of this statistic is then approximated by randomization tests to obtain empirical *P* values.[24] We implemented 10,000 permutations of case–control status to calculate empirical *P* values. Within haplotype configurations that had significantly different case–control haplotype frequency distributions, we examined χ^2 statistics comparing the frequencies of individual haplotypes between cases and controls, referenced against all other haplotypes in that configuration. The null distributions of these statistics were also approximated by randomization tests using 10,000 permutations of case-control status to derive empirical *P* values.[24]

Subject-level haplotypes were reconstructed using PHASE.[25] PHASE assumes a coalescent prior and implements Gibbs sampling (a Bayesian methodology) to infer haplotypes using unphased genotype data. The prior expectation included in this model is that unresolved haplotypes (subjects who are ambiguously phased) tend to be similar to known haplotypes. This method has been shown to reduce error rates by greater than 50% in some circumstances.[25] We excluded subjects if 50% or more of the markers had missing genotype data, or if any of the loci-specific phase probabilities dropped below 90%. Odds ratio (OR) estimates and 95% confidence intervals (CIs) compared subjects who carried the index haplotype with all other subjects. These were estimated in 2-by-2 contingency tables within strata of race.

TABLE 1. Allele Frequencies of Proinflammatory Cytokine Polymorphisms

SNP Identifiers			Minor Allele Frequency	
Polymorphism	**Alternative Identifiers**	**dbSNP ID**	**African-American**	**Whites**
IL1α(+4845) G>T	A114S	rs17561	T = 0.17	T = 0.27*
IL1α(IVS5–109) A>C		rs2071374	C = 0.32	C = 0.28*†
IL1β(+3954) C>T	F105	rs1143634	T = 0.13	T = 0.23
IL1β(−581) C>T		rs1143627	T = 0.38	C = 0.37
IL1β(−1061) C>T		rs16944	C = 0.45	T = 0.35
IL2(−385) G>T		rs2069762	G = 0.08	G = 0.29*†
IL6(−237) C>G	*(−174)*	rs1800795	C = 0.06	C = 0.42
TNF(−488) G>A	*(−308)*	rs1800692	G = 0.25	A = 0.17
LTA(IVS1+90) G>A		rs909253	A = 0.14	G = 0.32
LTA(IVS1–82) G>C		rs746868	A = 0.49	C = 0.40

*Borderline violation of Hardy-Weinberg Equilibrium in spontaneous preterm birth controls (*P* value = 0.03–0.05).

†Borderline violation of Hardy-Weinberg Equilibrium in SGA controls (*P* value = 0.03–0.05).

Semi-Bayesian hierarchical logistic regression was used to obtain the OR estimate and 95% CI for the main effect of each cytokine polymorphism for SGA and spontaneous preterm birth, with separate models for African-American and white subjects. Hierarchical regression greatly improves the accuracy of unstable estimates, especially when multiple exposures (in this case, polymorphisms) are under study, and individual cell sizes are small.[26] Our first-stage logistic regression model regressed case-status on the individual cytokine polymorphisms. Dichotomous versions of polymorphisms were created comparing carriers to noncarriers, and all models were adjusted for an ever/never version of smoking during pregnancy. Our second-stage linear regression model regressed the SNP β-coefficients on covariates thought to predict their magnitude. We included 2 second-stage covariates that grouped polymorphisms by immunologic pathways. The first covariate assigned polymorphisms in proinflammatory cytokines a "1" and polymorphisms in anti-inflammatory cytokines "0"; the second covariate did the opposite. Consequently, we assumed the target parameters for cytokine polymorphisms within the same immunologic pathway were randomly sampled from a common underlying distribution with an unknown mean.[27] Residual variation unexplained by the second-stage covariates was captured in the independent random variable, δ, which has mean 0 and variance τ.[2] We assumed with 95% certainty that the OR for each SNP, after adjusting for the second-stage covariates, would fall within a 10-fold range (τ^2 of 0.35). We conducted sensitivity analyses to determine the degree to which our assumptions about residual variation affected our estimates (data not shown). All models were adjusted for smoking during pregnancy. Single locus effects were also estimated in 2-by-2 contingency tables, stratified by race.

We implemented a 3-tiered analysis approach: (1) we applied omnibus likelihood ratio tests to identify regions that may contain one or more disease-predisposing alleles; (2) we reconstructed subject-level haplotypes and obtained effect estimates comparing subjects who carried at least one copy of the index haplotype with all other subjects; (3) we examined SNP associations within strata of race for haplotypes that were strongly associated with the odds of the outcome in a hierarchical framework that adjusted for all polymorphisms, their immunologic pathway, and smoking during pregnancy. We also analyzed SNPs in 2-by-2 contingency tables, stratified by race.

Finally, we examined a potential gene–environment interaction between bacterial vaginosis infection in the 24th-29th gestational week and the presence of a proinflammatory high-risk allele on spontaneous preterm birth. Breslow-Day tests of homogeneity were examined for statistical evidence of deviation from mutliplicativity. The Interaction Contrast Ratio was examined for evidence of deviation from additivity.[28,29]

RESULTS

Spontaneous Preterm Birth

IL1α and *IL1β*

There were no differences in the overall *IL1β* haplotype frequency distribution between cases and control subjects among either whites or African-Americans. Similarly, there were no differences in African-Americans for the *IL1α* haplotype configuration. (Whites were excluded from haplotype analyses of *IL1α* due to a modest departure from Hardy-Weinberg equilibrium; Appendices 3 and 5). White women who carried the +3954C/−581C/−1061T haplotype had 1.7 (0.9–3.2) times the risk of spontaneous preterm birth, and subjects who carried the +3954C/−581T/−1061C haplotype had 2.1 (0.9–5.2) times the risk of spontaneous preterm birth. In hierarchical regression, white carriers of the *IL1β(−1061)T* or *IL1β(−581)C* alleles were not at substantially increased risk of spontaneous preterm birth (for *IL1β(−1061)*, 1.4 [0.6–3.5]; for *IL1β(−581)*, 1.2 [0.5–3.0]). White carriers of *IL1α(+4845)T* were at higher risk (1.8; 0.9–3.7; Fig. 2 and Appendix 4). The risk associated with carrying any of the *IL1* polymorphisms did not appear elevated in hierarchical regression among African-Americans (Fig. 2 and Appendix 6).

IL2

Neither white nor African-American carriers of *IL2(−385)G* had increased risk of spontaneous preterm birth in hierarchical regression (for white women, 1.0 [0.5–3.0]; for African-American women, 0.7 [0.3–1.6]; Fig. 2 and Appendices 4 and 6).

IL6

Both white and African-American carriers of *IL6 (−237)C* had similarly increased risk of spontaneous preterm birth (for white women, 1.8 [0.8–3.6]; for African-American women, 1.6 [0.7–3.8]) (Fig. 2 and Appendices 4 and 6). The frequency of the *IL6* variant allele in African-American women is low (6%; Table 1).

TNF and *LTA*

Polymorphisms in the *TNF* and *LTA* genes were strongly associated with one another (for all pairwise comparisons in African-Americans and whites, $D' \geq 0.98$), and were therefore considered as part of a common haplotype configuration. Among white mothers, haplotype frequency distributions differed between spontaneous preterm cases and control subjects (Table 2). Differences in the overall frequency distribution appeared to be driven by the AGG and GAG haplotypes (for the AGG haplotype, 1.5 [0.8–2.6]; for the GAG haplotype, 0.6 [0.3–1.0]). Carriers of the GAC haplotype were at increased risk of spontaneous preterm birth (1.6; 0.9–2.9), although the haplotype frequency was not substantially different between cases (0.42) and control subjects (0.38). White subjects who carry the *TNF(−488)A* or *LTA(IVS1−82)C* variants also had increased

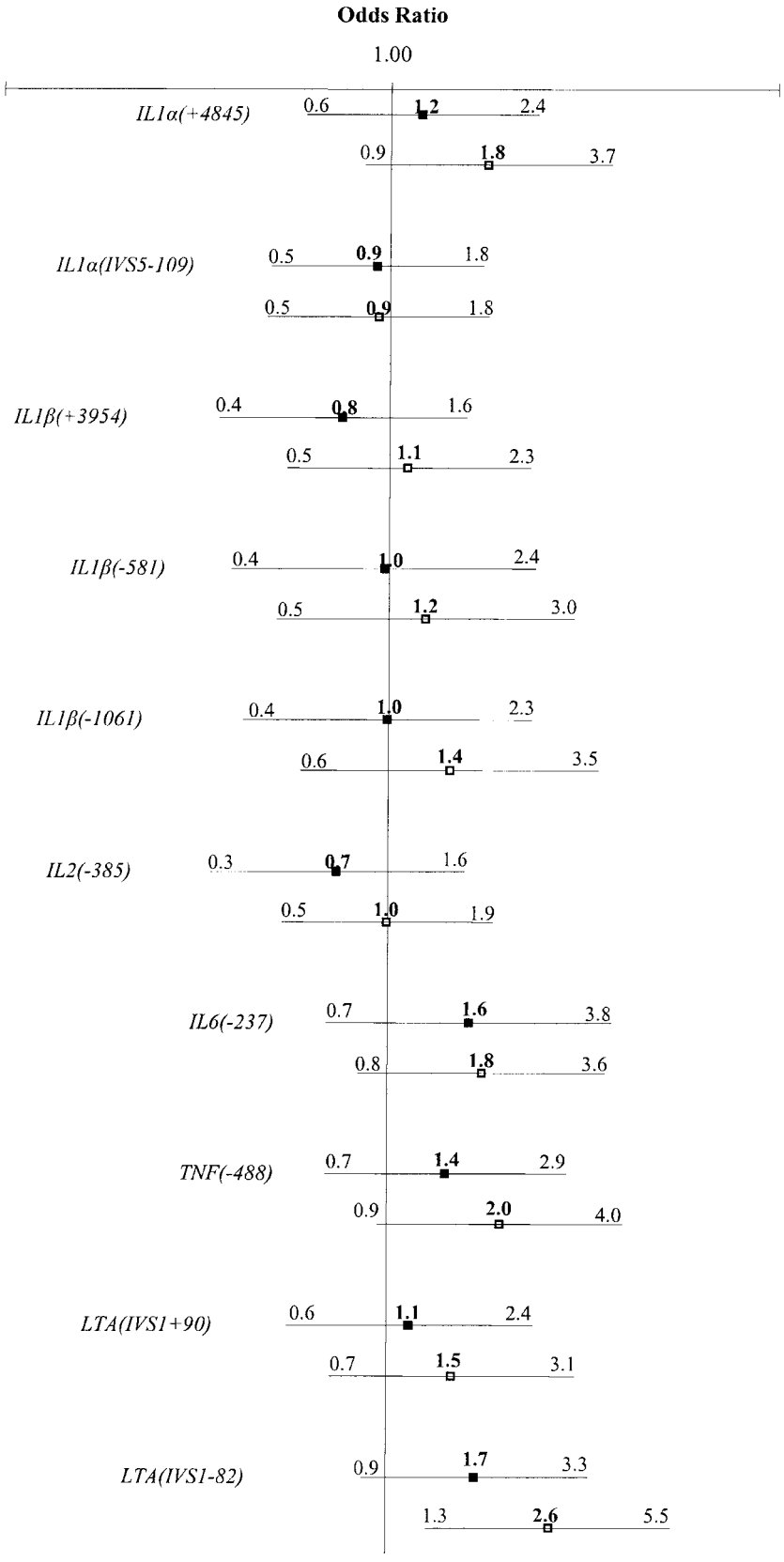

FIGURE 2. Risk of spontaneous preterm birth for proinflammatory cytokine polymorphisms in semi-Bayesian hierarchical logistic regression models that included both pro- and anti-inflammatory cytokine polymorphisms and were adjusted for smoking and the inflammatory mechanism of the cytokine, stratified by maternal self-reported race. Odds ratios and 95% confidence intervals reflect the effect of carrying a given polymorphism on risk for spontaneous preterm birth. Filled squares represent the point estimates for African-American mothers; open squares represent the point estimates for white mothers.

TABLE 2. Haplotype Analysis of Risk of Spontaneous Preterm Birth in Whites

			Haplotype Frequency Estimation (EM)[23]					Haplotype Reconstruction (PHASE)[25]	
TNF(−488)	*LTA(IVS1+90)*	*LTA(IVS1–82)*	Overall*	Case	Control	χ^2	*P* value†	OR‡	(95% CI)
A	G	G	0.16	0.24	0.15	3.92	0.01	1.5	(0.8–2.6)
G	A	C	0.39	0.42	0.38	2.21	0.24	1.6	(0.9–2.9)
G	A	G	0.29	0.20	0.31	5.93	0.01	0.6	(0.3–1.0)
G	G	G	0.16	0.14	0.16	4.03	0.50	1.1	(0.6–2.0)
Omnibus Likelihood Ratio Test:						9.18	0.05		

*Haplotypes included in table if they have a frequency of at least 5% among white women. Complete tables are available from the authors by request.
†*P* values based on 10,000 permutations and represent the comparison of individual haplotype frequencies between cases and controls.
‡Odds Ratio (OR) and 95% Confidence Interval (CI) calculated using subject-level haplotype assigments from PHASE comparing carriers of a given haplotype to noncarriers of that haplotype.

risk of spontaneous preterm birth in hierarchical regression (for *TNF(−488)A*, 2.0 [0.9–4.0]; for *LTA(IVS1−82)C*, 2.6 [1.3–5.5]; Fig. 2 and Appendix 4). Additionally, subjects who carry the haplotype that contains none of the variant alleles, GAG, had 40% reduced risk of spontaneous preterm birth (0.6; 0.3–1.0).

There was no difference in haplotype frequency distributions between spontaneous preterm cases and control subjects in African-American women (Appendix 5). However, the pattern of single locus effect estimates was similar in direction to whites, but with smaller magnitude (Fig. 2 and Appendix 6).

Gene–Infection Interaction Between Proinflammatory Polymorphisms and Bacterial Vaginosis Infection

In white women, there were no significant multiplicative interactions between inflammatory cytokine polymorphisms and bacterial vaginosis infection; *TNF(−488)A* and *LTA(IVS1+90)G* both deviated from additivity, although in an unexpected direction (interaction contrast ratios of −2.8 and −3.7 respectively). In African-American women, each of the joint effects of *IL1β(+3954)T, IL6(−237)C*, and *TNF(−488)A* and bacterial vaginosis infection were both greater than multiplicative and greater than additive, yielding interaction contrast ratios ≥3.0 and Breslow-Day *P* values ≤ 0.10. In particular, carriers of *IL6(−237)C* with bacterial vaginosis had greater than 4-fold the risk of spontaneous preterm birth compared with women who carried the variant but were not infected (4.4; 1.2–16.4). Similarly strong effects on preterm birth were found among carriers of *TNF(−488)A* (2.8; 0.9–9.1) and *IL1β(+3954)T* (2.8; 0.9–8.6). (Complete interaction tables available from the authors by request.)

SGA

IL1α and *IL1β*

There were no differences in *IL1β* or *IL1α* haplotype frequency distributions between cases and controls for either white or African-American women (Appendices 7 and 8). White women who carried the *IL1β(+3954)C/IL1β (−581)C/IL1β(−1061)T* haplotype had 40% reduced risk of SGA (0.6; 0.3–1.0). Both the *IL1β(−581)C* and *IL1β(−1061)T* variants, contained in the CCT haplotype, have hierarchical regression estimates below 1.0 (for *IL1β(−581)C*, 0.7 [0.3–1.7]; for *IL1β(−1061)T*, 0.6 [0.2–1.3]; Fig. 3 and Appendix 4). There were no notable haplotype or hierarchical SNP effects among African-American women.

IL2

White women who carried the *IL2(−385)G* allele had modestly increased risk of SGA (1.4; 0.8–2.5) (Fig. 3and Appendix 4). This association was more pronounced in 2-by-2 contingency tables (1.6; 1.0–2.6). There was no association in African-American women (Fig. 3 and Appendix 6).

IL6

IL6(−237)C was not associated with SGA in either whites (0.8; 0.4–1.4) or African-American women (1.0; 0.4–2.5; Fig. 3 and Appendices 4 and 6).

TNF and *LTA*

There were no differences in haplotype frequency distributions in either African-Americans or white women, and no individual haplotypes were strongly associated with SGA (Appendices 7 and 8).

DISCUSSION

To assess the contribution of genetic variation in proinflammatory cytokines to the etiology of spontaneous preterm birth and SGA, it is critical to study gene pathways because cytokines have pleotropic effects on host response. In this regard, the study of complex conditions, such as spontaneous preterm birth and SGA, should focus on critical sets of genes, both upstream and downstream responders. The temporal

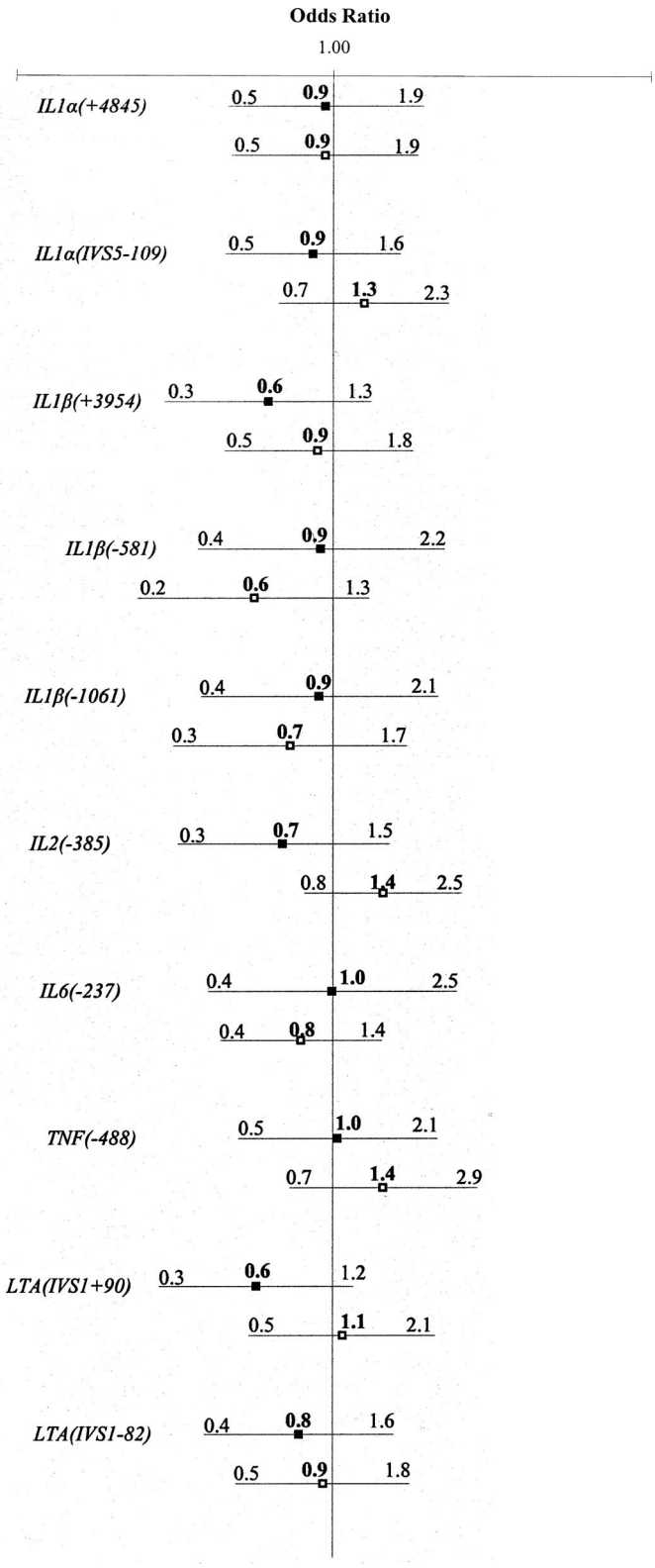

FIGURE 3. Risk of SGA for proinflammatory cytokine polymorphisms in semi-Bayesian hierarchical logistic regression models that included both pro- and anti-inflammatory cytokine polymorphisms and were adjusted for smoking and the inflammatory mechanism of the cytokine, stratified by maternal self-reported race. Odds ratios and 95% confidence intervals reflect the effect of carrying a given polymorphism on risk for SGA. Filled squares represent the point estimates for African-American mothers; open squares represent the point estimates for white mothers.

alteration of cytokine levels in response to multifactorial challenges (eg, infection and toxins) could prove to be critical in the development of spontaneous preterm birth or SGA. Likewise, small changes in expression over time could be accentuated in the setting of gestation, because pregnancy is characterized by a delicate balance between normal immune function and continual antigen challenge.

We undertook an investigation of 10 polymorphisms in proinflammatory cytokines (*IL1α, IL1β*, *IL2*, *IL6*, *TNF*, and *LTA*) and risk of spontaneous preterm birth and SGA in a case–control study, nested within a well-defined prospective cohort of pregnant women. A companion report contains the second part of our investigation, in which we describe the relationship of polymorphisms in anti-inflammatory cytokines with spontaneous preterm birth and SGA.[17] The polymorphisms we included were either identified from previous studies or included because their minor allele frequency was greater than 5% and prior in vitro data suggested a functional effect. Because allele and haplotype frequencies, pairwise linkage disequilibrium estimates, and risk of spontaneous preterm birth differ between races, all of our analyses were stratified by maternally self-identified race; however, we cannot exclude the possibility of residual confounding by race within these groupings. This study was limited by a small sample size once stratified by race.

It has been postulated that perturbations of the inflammatory response could contribute to the etiology of spontaneous preterm birth.[1,3] Amniotic fluid concentrations of TNF-α and IL-6 have been found to be elevated in association with preterm labor and birth.[1,30,31] Inflammatory cytokines TNF-α, IL-1β, and IL-6 stimulate prostaglandin synthesis in the amnion, chorion, and decidual tissue, which contributes to the initiation of labor. Furthermore, TNF-α is known to induce apoptosis, and high levels of TNF-α could damage the placenta either directly, or by activating natural killer cells, lymphokine activated killer cells, and macrophages,[32] which in turn weaken fetal membranes. TNF-α also stimulates the production of matrix metalloproteinase enzymes, which degrade collagen,[31,33] a major constituent of membranes. However, increased concentrations of inflammatory cytokines are also linked to term deliveries and may be a normal component of the body's preparation for parturition.[31,34,35] Some cases of preterm birth could reflect an early activation of these components normally associated with delivery, and some could result from infection and genetic predisposition combined.

Previous studies have examined the relationship between *TNF(−488)A* and mother's susceptibility to spontaneous preterm birth.[6,7,11,12,14] The polymorphisms we included in the *TNF* region have functional consequences on gene transcription; specifically, the *TNF(−488)A/LTA(IVS1+90)* G haplotype has been linked to increased production of TNF-α and LTA relative to all other haplotypes.[36,37] This haplotype has been associated with autoimmune, allergic, and infectious diseases.[38–41] We found that the AGG, GAC, and GAG haplotypes were strongly associated with increased or decreased odds of spontaneous preterm birth in whites. Currently, there are no data indicating that increased *TNF* or *LTA* expression can be attributed to the *LTA(IVS1−82)C* allele. However, carriers of the GAC haplotype or the *LTA(IVS1−82)C* allele also had increased risk of spontaneous preterm birth, which may point to linkage disequilibrium between *LTA(IVS1−82)C* and another causal variant. We have accounted for only a small proportion of the variation within *TNF* and *LTA*, which may explain the lack of consistency in haplotype results between white and African-American women—the latter possessing less linkage disequilibrium across markers and therefore greater haplotype diversity. Nonetheless whites and African-Americans had similar patterns in hierarchical regression effect estimates.

Both African-American and white carriers of *IL6(−237)C* had increased risk of spontaneous preterm birth. The hierarchical regression estimates were of approximately the same magnitude and precision even though the *IL6(−237)C* variant occurs at substantially lower frequency in the African-American subgroup. Circulating IL-6 levels vary inversely with progesterone during a normal menstrual cycle.[42] Additionally, a recent clinical trial in a high-risk population found that weekly intramuscular injections of 17 alpha-hydroxyprogesterone caproate reduced risk of preterm birth by approximately one third.[43] Not surprisingly, the levels of IL-6 in maternal serum, amniotic fluid, vaginal fluid, and placenta have been reported to increase during normal labor, with even higher levels observed in women undergoing preterm labor.[44] IL-6 secretion is regulated by a complex haplotype, including an A_nT_n site upstream of -237.[45] However, in the *IL6* promoter, the predominant haplotype including the *IL6(−237)G* variant has been shown to account for higher *IL6* expression. We found that carriers of the *IL6(−237)C* variant were at increased risk of spontaneous preterm birth, which, counterintuitively, suggests that those subjects who produce less IL-6 (possibly leading to higher levels of progesterone) have higher risk of preterm birth. This finding should be validated in a larger study.

Finally, polymorphisms in *IL1*α and *IL1*β were modestly associated with spontaneous preterm birth in white women. Although carriers of *IL1*α*(+4845)T* were at increased risk, this polymorphism violated Hardy–Weinberg in our white subgroup. However, *IL1*α*(+4845)T* is believed to be functional; the G to T substitution results in an amino acid change from alanine to serine at residue 114. It has been hypothesized that this may affect proteolytic processing of proIL-1α due to its proximity to a protease cleavage site, causing proIL-1α to build up inside the cell and leading to less circulating IL-1α.[46]

We found preliminary evidence for gene–environment interactions with bacterial vaginosis infection in African-American women. African-American women who carried any proinflammatory SNP and were negative for bacterial vaginosis at 24–29 weeks' gestation had very low risk of spontaneous preterm birth, whereas those who both carried *IL1*β*(+3954)T, IL6(−237)C*, and *TNF(−488)A* and were positive for bacterial vaginosis had substantially increased risk. This increase was greater than the additive and the multiplicative benchmarks. Our results lend support to the hypothesis that inflammation may be a critical factor in the etiology of preterm birth; however, the nature of this relationship is still unclear.

The evidence linking cytokines to SGA has been mixed. Both pro- and anti-inflammatory cytokine-favored environments have been implicated.[47–50] We examined the role that genetic variants in proinflammatory cytokines play in the etiology of SGA and found only a borderline association for *IL2(−385)G* limited to whites. This finding suggests that this pathway might not be critical for SGA development. Although we chose frequent SNPs and haplotypes to cover the most likely candidate SNPs in the genes of interest, it is still possible that additional variants in these genes could be associated with SGA.

The relationship between proinflammatory cytokine SNPs and spontaneous preterm birth is complex. Only the *TNF/LTA* haplotypes were associated with spontaneous preterm birth in both African-Americans and whites. The relationship between proinflammatory cytokines and spontaneous preterm birth may well be modified by the presence of bacterial vaginosis infection during pregnancy, but more detailed infection data are needed to examine this hypothesis thoroughly.

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